Carcinogenesis vol.14 no.1 pp.151–154, 1993

SHORT COMMUNICATION

Smoking-related DNA adducts: ^{32}P-postlabeling analysis of 7-methylguanine in human bronchial and lymphocyte DNA

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7-methylguanine DNA adducts were determined in macroscopically normal bronchial specimens and peripheral blood lymphocytes of 20 patients undergoing pulmonary surgery. A recently developed ^{32}P-postlabeling assay was applied with anion exchange chromatography as an adduct enrichment method. The material consisted of 13 smokers and 7 non-smokers. The mean bronchial 7-methylguanine levels of 11 smokers and 6 non-smokers were 17.3 and 4.7 adducts/10^7 nucleotides. In lymphocyte DNA, the respective mean levels were 11.5 and 2.3 adducts/10^7 nucleotides. The bronchial DNA adduct levels in smokers were statistically higher than those in non-smokers. Among 5 smokers, for whom both bronchial and lymphocyte DNA was available, 7-methylguanine levels correlated in the two tissues (r = 0.77).

Tobacco is considered to be one of the major causes of cancer (1). Many of the several thousand chemicals present in tobacco smoke are carcinogenic (2). Many of these carcinogens are also known to form covalent DNA adducts (e.g. polycyclic aromatic hydrocarbons, aromatic amines, *N*-nitrosamines (3). The ^{32}P-postlabeling method has shown to be very suitable for detecting large, non-polar DNA adducts (4), and most human studies on tobacco-related DNA adducts, have focused on the polycyclic aromatic hydrocarbon-type of adducts (5–19). A potential role of tobacco-specific *N*-nitrosamines in smoking-related cancers has been proposed (20). The main tobacco-specific *N*-nitrosamines, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) and *N'*-nitrosonornicotine (NNN), NNK being a methylating agent, are carcinogenic in animals, and smokers are estimated to be exposed to these compounds at significant levels (20). In a previous study the relationship between O^6-methylguanine in placental DNA and smoking was investigated (21). The present study is the first attempt to show by the ^{32}P-postlabeling assay 7-methylguanines in human target and non-target tissue DNA in respect to smoking. DNA adducts were measured in bronchial specimens and peripheral blood lymphocytes of smokers and non-smokers.

Sections of macroscopically normal bronchial tissue and blood were obtained from patients undergoing pulmonary surgery in the Thoracic Surgical Clinic of Postgraduation Medical School, Budapest, Hungary. Most of the 20 patients were carcinoma patients, except for patients no. 18 and 20 (Table I). Based on hospital records the patients were divided into smokers and non-smokers, i.e. never-smokers; the mean ages of these groups were 49.7 and 60.1 years respectively.

Lymphocytes were isolated by centrifugation in Ficoll Paque (14). DNA isolation from bronchial specimens and peripheral blood lymphocytes is described elsewhere (7). The procedure for DNA digestion, adduct enrichment with anion exchange chromatography and ^{32}P-postlabeling of human DNA (13–19 μg) is described elsewhere (22,23). In the present study the labeled samples were applied to 10 × 10 cm TLC plates (Maherey Nagel) and developed in the first dimension with 0.1 M ammonium format, pH 5.3, and in the second dimension with 0.1 M LiCl (Figure 1). Student's *t*-test was used for statistical evaluations as the data were normally distributed. Different tests were applied when variances in the data sets were equal or nonequal.

Figure 2 shows chromatographic patterns of 7-methylguanine analyses of bronchial and lymphocyte DNA isolated from smokers and non-smokers. Individual 7-methylguanine adduct determinations are based usually on 3 separate ^{32}P-postlabeling analyses. The mean bronchial adduct levels in 11 smokers and 6 non-smokers were 17.3, and 4.7 7-methylguanines/10^7 nucleotides respectively (Figure 3). The corresponding values in lymphocyte DNA were 11.5 and 2.3 7-methylguanines/10^7 nucleotides respectively, in 7 smokers and 3 non-smokers. Statistical analysis showed that the mean bronchial adduct levels of smokers were significantly higher than the means of non-smokers. In lymphocyte DNA the difference between smokers and non-smokers was of borderline significance ($P = 0.055$). When 7-methylguanine levels in bronchial and lymphocyte DNA, obtained from the same smokers, were compared, a correlation was observed (Figure 4). Unfortunately, the correlation (r = 0.77) was based on only 5 individuals from whom both tissue samples were available (cf. Table I). There was no correlation in bronchial tissue or in lymphocytes between the adduct levels and the daily consumption of cigarettes (data not shown).

Several reports have demonstrated, by using mainly the ^{32}P-postlabeling assay, the presence of aromatic adducts in smokers' bronchus or lung tissue (5–21). However, tobacco smoke contains more than 4 different compounds suggested to be carcinogenic (24). Among these, the main tobacco-specific *N*-nitrosamines, NNK and NNN, are both found in relatively high levels in mainstream and sidestream smoke and also in unburned tobacco (20). A potential role of NNK and NNN in the induction of cancers of the lung and esophagus has been proposed. Hydroxylation reactions are suggested as the main metabolic pathways for tobacco-specific *N*-nitrosamines, resulting at least in the case of NNK in the formation of methyl diazonium hydroxide, capable of DNA methylation (20).

DNA methylation at the O^6- and N-7 positions of guanine by tobacco-specific *N*-nitrosamines has mainly been identified in the target tissue of experimental animals (25,26). A correlation between O^6-methylguanine levels, suggested promutagenic

Table I. Descriptive data of the patients whose bronchial and blood samples were analysed

Patient no[a]	Age Time since stopping smoking[c]	Diagnosis[b]	Years smoked	Smoking history Cigarettes/day
Smokers				
1 (m)	62	S	42	10
2 (m)	53	S	35	3
3 (m)	48	S	33	20
4 (m)	47	A	32	8
5 (m)	67	A	50	20
6 (m)	64	AN	50	60
7 (m)	38	S	20	25
8 (m)	58	S	45	20
9 (f)	51	SC	32	20
10 (f)	39	L	33	10
11 (f)	42	A	20	6
12 (f)	40	AN	26	16
13 (f)	37	A	12	7
Non-smokers				
14 (m)	65	A		
15 (m)	69	S		
16 (f)	59	A		
17 (f)	53	A		
18 (f)	64	pneumonitis		
19 (f)	66	A		
20 (f)	45	cystadenoma		

[a]m = male; f = female.
[b]A, adenocarcinoma; AN, anaplastic carcinoma; L, large cell carcinoma; SC, small cell carcinoma; S, squamous cell carcinoma.

adducts (27), and the formation of pulmonary neoplasia has been shown in rats (28). In a few studies methylated DNA adducts (O^6-methylguanines) have been analysed in human DNAs from different sources (21,29–31). In the present study we analysed 7-methylguanines in human DNA by using the ^{32}P-postlabeling assay with a recently developed anion exchange chromatography as an adduct enrichment method (22,23). Patients undergoing pulmonary surgery offered an opportunity to compare the presence of DNA adducts in bronchial tissue and peripheral blood lymphocytes in relation to smoking.

The present data demonstrated statistically significant differences between smokers' and non-smokers' bronchial DNA: smokers had ~5-fold higher mean adduct levels than non-smokers respectively. In the present study, the mean adduct level in smokers as compared to non-smokers was almost significant in lymphocytes. Our recent results with healthy volunteers also showed a difference between smokers and non-smokers (23).

Interindividual variations in the level of adducts were large, 27–46-fold, in bronchial DNA of smokers and non-smokers respectively; in the lymphocyte DNA the adduct levels varied somewhat less. The adduct values obtained here are in general in the range of our previous results for white blood cells (22,23). Shields *et al.* (32) reported, using combined high-performance liquid chromatography/^{32}P-postlabeling, levels of 7-methylguanines comparable to the present bronchial levels in lung samples of 5 trauma victims, who were smokers.

The adduct levels in bronchial tissue of the individual smokers correlated with the lymphocyte adduct levels ($r = 0.77$). This is an important observation, although based on 5 individuals only, because it shows for the first time that a specific adduct in lymphocytes is correlated with the adduct level in the target tissue. In a previous study on smokers van Schooten *et al.* (19) reported no correlation between total white blood cell and lung adducts,

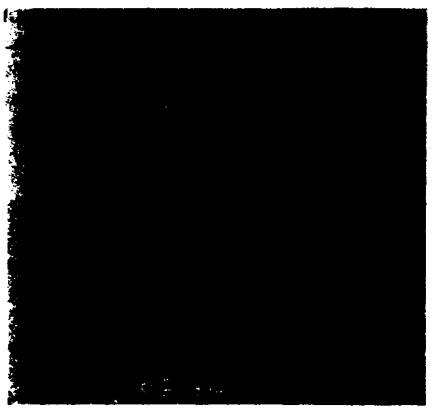

Fig. 1. An autoradiogram of a polyethylene imine-cellulose TLC plate of 7-methylguanines enriched by anion exchange chromatography. DNA (13–19 μg) isolated from bronchial specimens or peripheral blood lymphocytes were labeled with [γ-^{32}P]-in the presence of T4 polynucleotide kinase for 1 h after which 3'-phosphates were removed with nuclease P1. 7-Methylguanines were separated in a two-dimensional thin-layer chromatography system using 0.1 M ammonium formate, pH 5.3, in the first direction. The plate was cut, as shown, before development of the second direction with 0.1 M LiCl. The exposure time of autoradiographic X-ray films (Kodak XAR-5) was 1–3 hours. For details of the procedure see (22,23).

but in fact the correlation among 8 current smokers was reasonable ($r = 0.52$). The analysis was based on no specific smoking-induced adducts.

We found no correlation between the bronchial nor lymphocyte adduct levels and the daily consumption of cigarettes. Several possible factors may modulate the individual levels of 7-methylguanines. Large interindividual differences in many

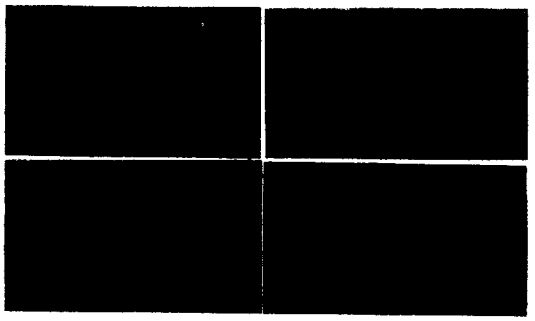

Fig. 2. The autoradiograms of polyethyleneimine-cellulose maps of 7-methylguanine adducts ^{32}P-postlabeled from bronchial (B) or blood lymphocyte (L) DNA of a non-smoker (1) and a smoker (4).

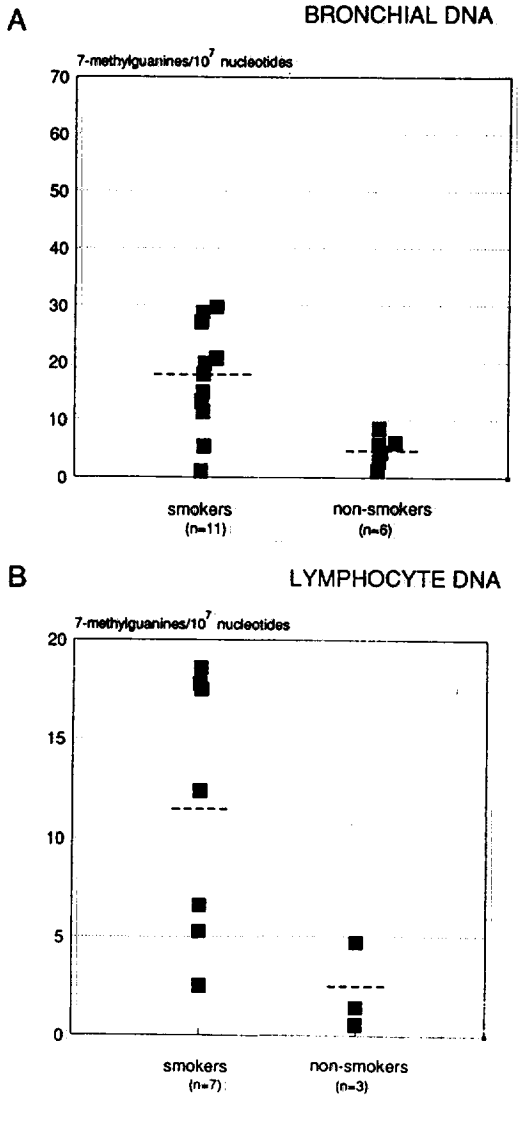

Fig. 3. Individual 7-methylguanine levels in bronchial (A) and lymphocyte (B) DNA of smokers and non-smokers. The differences between smokers and non-smokers were significant at $P < 0.01$ in bronchial samples and at $P = 0.055$ in lymphocytes.

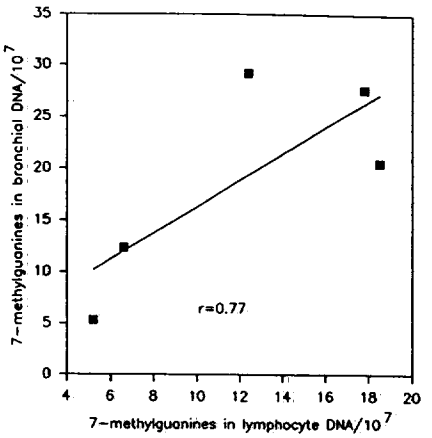

Fig. 4. Correlation of 7-methylguanine levels between bronchial and lymphocyte DNA from smokers.

pulmonary drug-metabolizing enzyme activities have been reported in humans (33,34). In tissue cultures and experimental animals the metabolism of different tobacco-specific *N*-nitrosamines and repair of methylated DNA demonstrated tissue and cell type specificity (35–37). Also little is known about spontaneous depurination *in vivo* and how it might vary between individuals. Lastly, the extent of endogenous methylation by e.g. S-adenosylmethionine and the formation of *N*-nitroso-compounds may also be confounding factors. Food is known to contain both precursors as well as catalysts and inhibitors of *in vivo* nitrosation (38).

In summary, the present study demonstrated the presence of 7-methylguanines in human target and non-target DNA in relation to smoking. In a small group of current smokers the levels of 7-methylguanine correlated between bronchial and lymphocyte DNA.

Acknowledgements

We thank Drs I.Vincze and S.Kostic for valuable collaboration.

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Received on September 2, 1992; revised on October 22, 1992; accepted on October 22, 1992